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1. Walton et al., Journal of clinical endocrinology and metabolism. 2001 August. Vol. 86, No. 8, pp. 3675-85.
2. Zarinan et al., Human Reproduction. 2001 august, vol. 16, No. 88, pp. 1611-1618.
3. Zambrano et al., Endocrine. April 1999. Vol. 10, No. 2, pp. 113-121.
4. Zambrano et al., Molecular human reproduction. August 1996, Vol. 2, No. 8, pp. 563-71.
5. Dahl et la., Journal of andrology. 1992, Jan-Feb. Vol. 13, No. 1, pp. 11-22.
6. Storring et al., Journal of Endocrinology. 1989. Vol. 123, No. 2, pp. 275-294.

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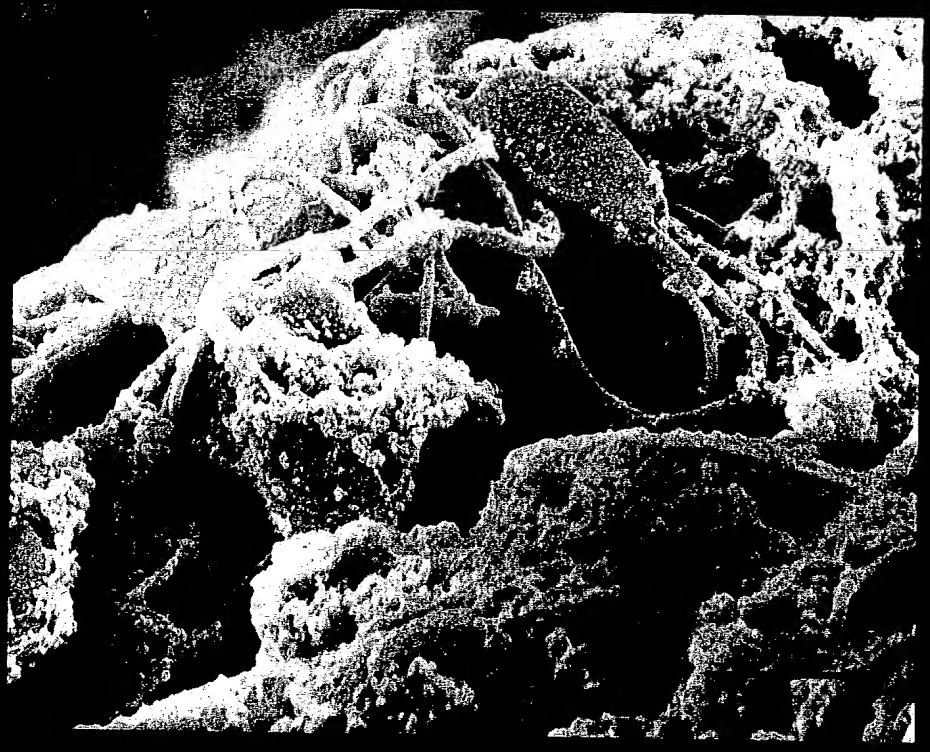

*Bio- and Immuno-hormone Analysis and Function*

*Macrophage Involvement in Testosterone Production*

*Proacrosin-acrosin in Rat Sperm*

Journal of Andrology, Vol. 13, No. 1, January/February 1992


# FSH Isoforms, Radioimmunoassays, Bioassays, and Their Significance

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**ABSTRACT:** Follicle-stimulating hormone (FSH) plays a central role in steroidogenesis and gametogenesis. In recent years, a great deal has been learned about the microheterogeneity of FSH using newly developed assay techniques. This review article will attempt to discuss the advantages and the limitations of recently developed and commonly used assay systems.
**J Androl 1992;13:11–22.**

Follicle-stimulating hormone (FSH), a pituitary gonadotropin, plays a central role in both testicular and ovarian reproductive processes. Synthesized in anterior pituitary gonadotrophs, FSH binds to gonadal receptors, leading to effects on steroidogenesis and gametogenesis. In recent years, a great deal has been learned about the microheterogeneity of FSH using newly developed assay techniques. These techniques have introduced new insights into the structure–function relationships of various FSH isoforms. However, before we fully appreciate these new insights, it is important to understand not only the advantages but also the limitations of recently developed and commonly used assay systems. This article will discuss the means by which structure–function relationships of FSH isoforms can be elucidated, and the possible interpretation of such findings.

## FSH Structure and Function

### Synthesis and Secretion

Follicle-stimulating hormone from all vertebrate species is a glycoprotein hormone containing two noncovalently linked dissimilar subunits, consisting of a common subunit designated α and a hormone-specific β subunit (Vaitukaitus et al, 1976; Sairam, 1983). The subunits are encoded by separate genes localized on different chromosomes. Beginning in the early 1970s, the primary amino acid sequence was determined. In recent years, the complementary DNAs and subunit genes have been isolated and characterized in several species (Pierce and Parsons, 1981; Gharib et al, 1990). The early developments that led to the postulation and eventual isolation of the subunit's structure have been reviewed in detail (Sairam, 1983; Saxena and Rathnam, 1976; Papkoff et al, 1973).

Follicle-stimulating hormone is synthesized and secreted by gonadotrophs of the anterior pituitary in response to various hypothalamic and gonadal factors. Gonadal proteins, such as inhibin and activin, influence the release of FSH. Inhibin increases in response to FSH, then feeds back on the pituitary to diminish FSH release in a classical endocrine feedback system (McLachlan et al, 1989). However, immuno-FSH levels do not always correlate with inhibin levels. These differences may be due to the effects of other gonadal proteins, or bioactive FSH levels may represent a more accurate assessment of the physiologic effects of gonadal proteins such as inhibin. On the other hand, activin has the ability to stimulate FSH release in cultured pituitary cells (Vale et al, 1986; Ling et al, 1986).

### Carbohydrate Structure

All glycoprotein hormones contain several asparagine-linked oligosaccharides (Kornfield and Kornfield, 1985). The primary structure of FSH consists of the polypeptide backbone and the oligosaccharide moiety. Follicle-stimulating hormone molecules from all species, including those from lower vertebrates, contain carbohydrate units covalently linked to the polypeptide (Sairam, 1983; Saxena and Rathnam, 1976; Papkoff et al, 1973; Chappel et al, 1983a). Carbohydrate chains are attached to the protein core by either an N- or O-linkage, but FSH has only N-linked carbohydrates (Baenziger and Green, 1988; Ryan et al, 1987; Pierce and Parsons, 1981). Follicle-stimulating hormone is glycosylated throughout the secretory process, from synthesis in the rough endoplasmic reticulum through the Golgi apparatus to various cellular and extracellular destinations. As seen in Figure 1 (Dahl and Hsueh, 1988), it has been postulated that the extent of glycosylation may depend on the type of stimulation.

The carbohydrate chains of FSH exhibit considerable

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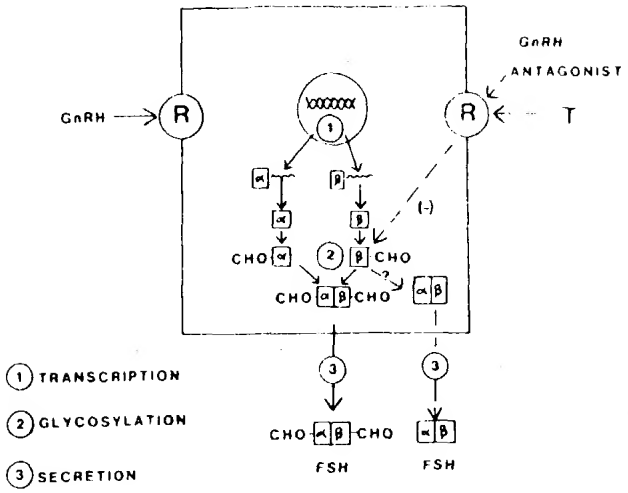


FIG. 1. Synthesis and processing of FSH (Dahl and Hsueh, 1988, with modifications).

variations in size and structure, ranging from mono- and disaccharide to branched oligosaccharides consisting of many monosaccharides (Baenziger and Green, 1988). Generally, the terminal carbohydrate moiety is either sialyated or sulfated (Ryan et al, 1987). The structures of the sialyated and sulfated asparagine-linked oligosaccharides are shown in Figure 2. The FSH α subunit of most species has more carbohydrate moieties than the β subunit; however, human FSH has two carbohydrate moieties on both subunits that have primarily sialic acid as the terminal sugars. Due to the immense diversity of the branching structures, the theoretical number of hormone isoforms is the product of the number of α forms and the number of β forms.

### Structure and Function

A brief outline of the current concept of the mode of action of FSH is depicted in Figure 3 (Dahl and Hsueh, 1988). The initial event is believed to be the binding of the hormone to the receptor, followed by the activation of adenylate cyclase, resulting in the accumulation of cyclic adenosine monophosphate (cAMP) and aromatase induction. This second messenger binds to the regulatory subunit of protein kinase A, releasing the catalytic subunit. This induces other events, leading to the amplification of the initial hormone signal, and culminating in steroidogenesis. It is believed that the protein and carbohydrate components of FSH are responsible for transmitting different physiologic signals to FSH target cells. The protein domain binds to specific FSH receptors on the plasma membrane, while the carbohydrate moiety in the subunit of FSH plays a critical role in coupling the hormone–receptor complex to the adenylate cyclase, and thus the transduction of the biologic signal (Ryan et al, 1987; Bahl et al, 1984).

There are a number of studies that have been concerned with the structure–function relationship of FSH (Chappel et al, 1983a; Sairam and Manjunath, 1982; Keutmann et al, 1982; Blum and Gupta, 1985; Peckham and Knobil, 1976). The essential role of sialic acid, which is present in FSH from all species (Manjunath et al, 1982), in maintaining the *in vivo* biologic activity of the hormone has been established. Removal of this sugar residue by neuraminidase treatment decreases the *in vivo* biologic activity of the hor-

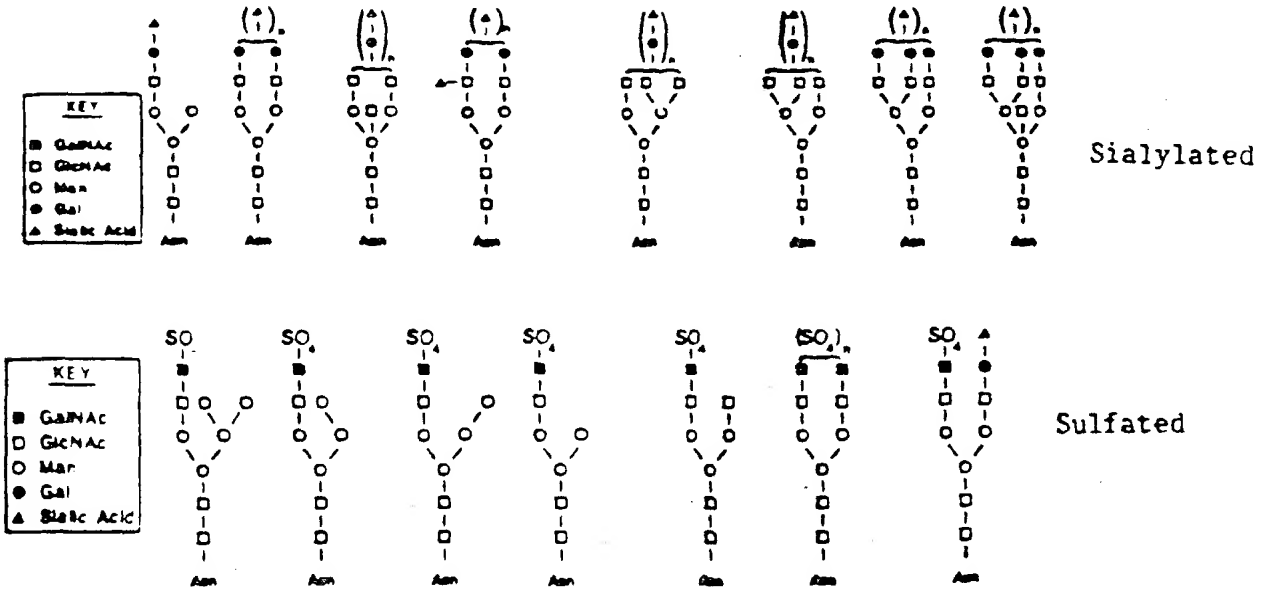


FIG. 2. Postulated carbohydrate moieties of FSH (Baezinger and Green, 1988, with modifications).

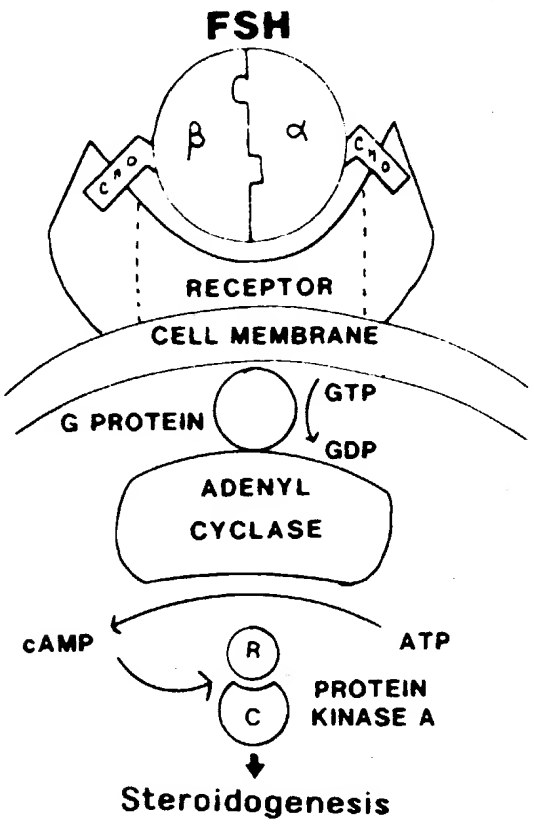


FIG. 3. Mode of FSH action on granulosa cell and Sertoli cell (Dahl and Hsueh, 1988).

mone due to its rapid elimination from the circulation (Ryle et al, 1970; Chappel et al, 1983b), but *in vitro* biologic response is retained (Ulloa-Aguirre et al, 1984, 1986). However, removal of additional sugars or chemical treatment have shown that the carbohydrate residues are not essential for binding to the receptor (Chappel et al 1983a; Moyle et al, 1978; McIlroy and Ryan, 1983).

### *Agonistic and Antagonistic Effects of FSH Analogs*

Both granulosa and Sertoli cells are regulated by pituitary FSH. Under appropriate conditions, granulosa cells, Sertoli cells, or seminiferous tubule suspensions can respond to the addition of FSH *in vitro* by the prompt accumulation of cAMP (Means et al, 1980). Manjunath and associates (1982) have demonstrated that specific removal of 80% of the carbohydrate moieties increased the receptor binding and immunologic activities of the hormone, as assessed by *in vitro* methods. Deglycosylated FSH also binds better than its intact counterpart (Calvo et al, 1986). In contrast, they have a greatly diminished capacity for stimulation of cAMP production (Fig 4; Manjunath et al, 1982). In addition, the deglycosylated preparations became potent and specific antagonists of the intact hormone in *in vitro* bioassays (Sairam and Manjunath, 1982; Keutmann et al, 1982; Manjunath et al, 1982; Liu et al, 1984).

## *FSH Radioimmunoassays, Radioreceptor Assays, and Bioassays*

### *Radioimmunoassays*

Following the development of a sensitive radioimmunoassay (RIA) for insulin, the technique was adapted for the measurement of FSH in humans (Midgley, 1967; Faiman and Ryan, 1967; Catt et al, 1972; Welsh et al, 1984) and rats (Ross and Gilman, 1980; Limbird, 1981). Several investigators were able to characterize changes in serum FSH in women during spontaneous and induced ovulatory cycles (Midgley and Jaffe, 1968; Ross et al, 1970). Since that time, serum levels of immunoreactive FSH have been characterized in humans during puberty, the menstrual cycle, menopause, and many endocrine states (Ross et al, 1970; Crowley et al, 1985).

Radioimmunoassay is convenient, specific, and sensitive, but due to the inherent nature of measuring only immunoreactive FSH, RIAs have major deficiencies. Since these assays are based on the immunoreactivity of the assayed material, discrepancies among assays using different antibodies have been reported (Taymore and Miyata, 1969; Diebel et al, 1973). Commercially available RIA kits show differences between each other, suggesting the antibodies differ for each kit (Fig 5, unpublished data; Jockenhovel et al, 1990). These kits see different molecules, and, therefore, give varying immuno-FSH levels for the same sample. Choosing an appropriate RIA standard (pituitary, urinary, etc.) adds additional complications. Due to the nonparallelism of competitive curves for FSH samples derived from different sources (eg, serum vs. urine), and the fact that most of the serum measurements were assayed against either pituitary or urinary standards, the quantitative aspect of the published data should be interpreted with caution (Fig 5; Jockenhovel et al, 1990; Albert et al, 1968; Ryan and Faiman, 1968). Although essentially all of the available data on FSH levels in body fluids have been obtained by RIA, these data do not necessarily reflect bioactivity. Some modified forms of FSH may not cross-react with the antibody but still retain their bioactivity, whereas some biologically inactive isoforms (eg, deglycosylated forms) may still bind the antibody.

### *Radioligand Receptor Assay*

The presence of specific receptors for FSH has been found exclusively in the granulosa cells of the ovary (Midgley, 1973) and in the Sertoli cells of the testes (Means et al, 1980). With this discovery, several investigators developed sensitive and specific radioligand receptor assays using

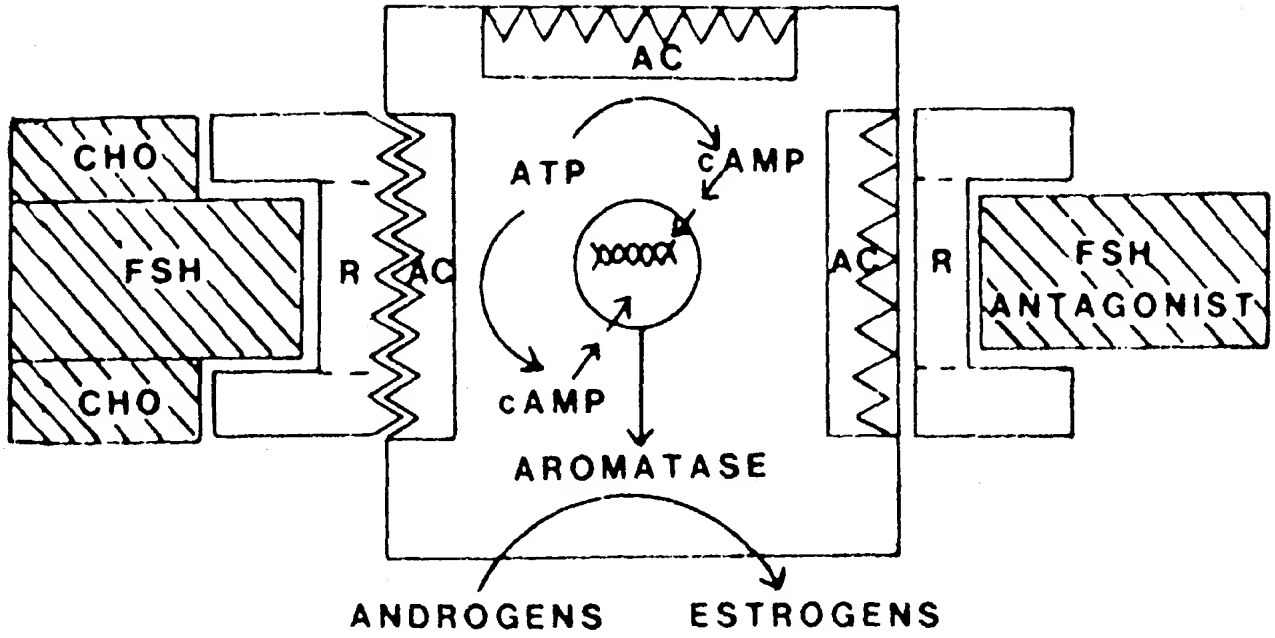


FIG. 4. Diagram suggesting potential effects of glycosylated versus deglycosylated FSH on receptor-mediated events.

granulosa cell preparations and purified rat testes homogenates (Cheng, 1975; Reichert and Bhalla, 1974). These methods have not gained popularity due to their low sensitivities, interference by serum inhibitors such as binding proteins and other unknown factors, and the difficulty in differentiating agonistic and antagonistic activities of FSH isoforms (Dahl et al, 1988; Reichert et al, 1991; Sluss et al, 1987).

### Bioassays

In Vivo: The classical *in vivo* FSH bioassays were based on FSH-induced increases in ovarian weight or follicle size in immature or hypophysectomized rodents (Steelman and Pohley, 1953; Brown and Wells, 1966). Some investigators also measured increases in testis weight of hypophysectomized rats (Simpson et al, 1950), or changes in testicular morphology of intact chicks. Also, "secondary" FSH responses, such as the increase in uterine weight of immature mice, have been used (Brown and Wells, 1966; Uberoi and Meyer, 1967). Among these assays, the Steelman and Pohley test is the most frequently used. In this assay, bioactivity of FSH is determined by treating immature female rats (21 to 22 days old) with 20 IU of human chorionic gonadotropin (hCG) plus test samples containing unknown quantities of FSH for 3 days. The ovarian weight is measured 72 hours after the first injection (Steelman and Pohley, 1953). This assay is superior to other methods because the addition of hCG in the injection protocol not only augments the ovarian weight increase (Simpson et al, 1950), but also minimizes the effect of contaminating luteinizing hormone (LH)-like materials in the assay samples. However, the mechanism by which hCG augments the FSH effect was not made clear until decades later. In general, *in vivo* bioassays are cumbersome and their sensitivities are too low to allow the detection of small amounts of FSH present in serum. An important advantage of the *in vivo* assays is their ability to take into account possible differences in the metabolic half-lives of the hormones (Wang, 1988).

In Vitro: Various *in vitro* bioassays for FSH have been developed in an effort to improve sensitivity and to develop

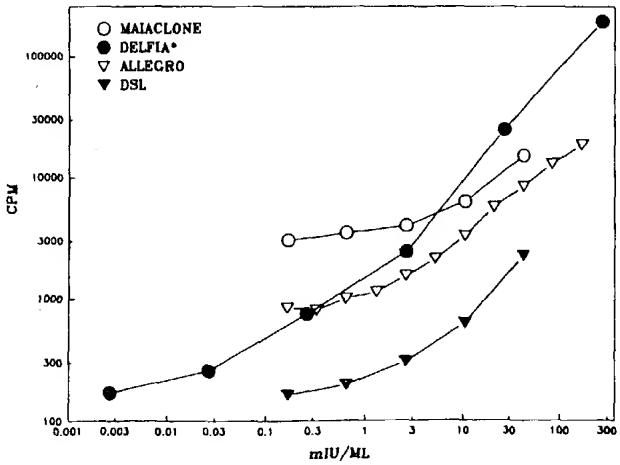


FIG. 5. Dose–response characteristics of the pituitary standard (LER-907) in four different immunoassays for FSH. *Delfia assay is colorometric and the Y scale is ten times stated.

tests that are easier to perform and whose results are more reproducible. These methods measure different biologic activities of FSH in various target tissues, including the uptake of radiolabeled thymidine into mouse ovaries (Ryle et al, 1970), and the incorporation of radiolabeled glucosamine into proteoglycans by porcine granulosa cells (Ax and Ryan, 1979). In addition, cAMP production by isolated rat seminiferous tubules was used as a bioassay for FSH (Ramachandran, 1983).

Beers and associates (1978) proposed an FSH bioassay based on the stimulation of plasminogen activator (PA) activity secreted by cultured granulosa cells from pregnant mare's serum gonadotropin-treated rats. However, subsequent studies have indicated that PA activity is increased by both FSH and LH (Wang and Leung, 1983; Ny et al, 1985). The only application of this method was the measurement of FSH content in conditioned media obtained from gonadotropin-releasing hormone (GnRH)-treated pituitary cell cultures (Beers et al, 1978). Because GnRH treatment alone increases PA activity (Wang, 1983; Hsueh et al, 1988), one is concerned about the validity of bioactive FSH measurement in samples containing both GnRH and pituitary hormones.

The most well defined and widely used *in vitro* bioassays for FSH are based on its capacity to stimulate aromatase activity in cultured Sertoli cells of male rats, as well as cultured granulosa cells of female rats. Follicle-stimulating hormone acts on granulosa and Sertoli cells to convert androgens to estrogens. The FSH effect on granulosa and Sertoli cell aromatases is dose-dependent and occurs at physiologic levels. The study of potential paracrine modulators (estrogens, androgens, and growth factors) that could enhance FSH action in granulosa and Sertoli cells led to the development of two *in vitro* bioassays. The combined action of enhancing hormones and factors resulted in assays that are highly sensitive to the aromatase-inducing function of FSH. These assays are hormone-specific and sensitive, and have been applied to the measurement of FSH bioactivity in serum samples from humans and diverse species (Van Damme et al, 1979; Padmanabhan et al, 1987; Dahl et al, 1989b).

The earlier *in vitro* bioassays suffered from low sensitivity and precision, and the addition of serum to all of the assays led to a further decrease in assay sensitivity and nonparallelism to the standard curve. The inhibitory effect of serum on the FSH response systems may be due to the presence of unidentified factors in serum that interfere with FSH binding to its receptor. Methods are now available to pretreat the samples and remove some interfering serum factors (Dahl et al, 1989a). By using small quantities of serum for a reduced period of time (24-hour incubation with the unknown samples after an initial preincubation of cells), parallelism to the standard curve was demonstrated in the Sertoli cell aromatase bioassay (Padmanabhan et al, 1987).

A distinct advantage of the granulosa cell and Sertoli FSH bioassays is the capability to measure FSH in all the mammalian species tested (Dahl et al, 1989b). In general, pituitary FSH from different species showed parallel dose–response curves in the present assay. In contrast to the nonparallelism observed in the RIA measurement of FSH from different sources, the granulosa cell aromatase bioassay (GAB) shows unique parallel dose–response curves for FSH preparations obtained from pituitary and urinary sources. However, due to the use of *in vivo* cultures, the present methodology does not take into account potential changes in *in vivo* metabolism of FSH. It is conceivable that certain potent FSH preparations in the *in vitro* assay may have a short half life *in vivo*, therefore exerting transient actions inside the whole organism.

### *FSH Microheterogeneity*

Marked disparities between bio- and immuno-estimates of both crude and purified hormone preparations have been observed (Albert et al, 1968; Ryan, 1969; Rosenberg et al, 1971). Data from Bogdanove's laboratory have suggested that heterogeneous populations of FSH are released by the pituitary in castrated rats, and the RIA data do not correspond with bioassay results (Diebel et al, 1973; Bogdanove et al, 1974). The bioactive to immunoreactive (B/I) ratio of test preparations changes with various physiologic conditions; androgens increase this ratio, whereas estrogens decrease it. Also, serum FSH from castrated rats had a shorter *in vivo* half-life than that from castrated androgen-treated rats (Bogdanove et al, 1974). In addition, variations in pituitary and serum FSH have been reported in rhesus monkeys after castration and hormonal replacement (Peckham et al, 1973; Peckham and Knobil, 1976). In direct contrast to the results derived from rats, serum and pituitary FSH from both ovariectomized and orchidectomized rhesus monkeys is characterized by a larger apparent molecular size, a higher B/I ratio, and a lower rate of disappearance from the circulation when injected into test rats. The role of estrogen in decreasing the B/I ratio was also demonstrated. The observed heterogeneity in the B/I ratio of FSH in these studies is believed to be due to variation in the sialic acid content of the hormone. These disparities between experiments require further elucidation. Because the proportion of the more basic bioactive forms of FSH increases with the onset of puberty in rats, Chappel and colleagues (1983a) suggested that the pituitary gland acquires the capacity of transforming acidic FSH species with lower bioactivity to their more potent counterparts, possibly by a pituitary neuraminidase. In addition, treatment with GnRH increases the relative proportion of the more basic form of pituitary FSH in the hamster (Galle et al, 1983). Studies on human pituitary FSH heterogeneity further suggested that the percentage of acidic forms of FSH increases with age, presumably due to changes in sialic acid content (Wide, 1985, 1987). Also,

estrogens are believed to induce the formation of more basic forms of FSH (Wide, 1982). However, the physiologic significance of this pleomorphism is now beginning to emerge due to the availability of sensitive bioassays to measure circulating, instead of pituitary, FSH levels.

## Clinical Studies

Despite the fact that FSH bioassays have been available, there are still large gaps in our knowledge regarding how specific treatment protocols result in discrepancies between serum FSH levels obtained by bioassay versus RIA. Changes in bio- and immuno-FSH suggest that processes such as biosynthesis and/or release and late stage glycosylation may be affected to alter the metabolism and circulating half-life. It is also possible that the tertiary structure may be altered, thereby affecting its ability to bind to the receptor but not to the antibody. Deglycosylated FSH has been shown to behave like an antagonist in *in vitro* bioassay systems. Recently, our studies demonstrated that administration of a GnRH antagonist resulted in the production of a circulating, naturally occurring antagonistic form of FSH (Dahl et al, 1988). Therefore, to understand the mechanisms by which various pathologic and physiologic states regulate FSH, it is essential to examine not only the levels of both bio- and immuno-FSH, but also whether differences in B/I ratios could be a result of alterations in their microheterogeneities, and where these alterations occur.

### GnRH Antagonist and Agonist Treatment

Synthetic derivatives of GnRH with agonistic and antagonistic properties have been developed and tested in several clinical studies, including suppression of prostatic cancer growth and the development of male contraceptives (Rivier et al, 1981; Linde et al, 1981; Labrie et al, 1986; Pavlou et al, 1986). Chronic administration of GnRH agonistic analogs leads to suppression of gonadal function through a complex mechanism of "pituitary desensitization." Serum immuno-LH and FSH levels are only modestly affected, whereas serum bio-LH levels are dramatically reduced. Consequently, the B/I ratio of LH, an index of the biopotency, decreased by approximately 90%. However, GnRH agonist treatment did not result in reliable suppression of spermatogenesis. When GnRH antagonists are administered to normal men, there is a decrease in both bio- and immuno-LH, and endogenous testosterone is decreased to castrate levels, but there is only a minimal decrease in immuno-FSH. Therefore, there are disparate effects of GnRH agonists and antagonists on LH B/I ratios and spermatogenesis. Most of the questions raised in the studies described above specifically relate to whether immuno-FSH data reflect bio-FSH, or if the disparate results are due to the differential regulation of circulating bioactive FSH. Therefore, we examined the effects of GnRH analogs on bioactive FSH levels in various endocrine states.

*GnRH Antagonist Treatment in Men:* Although GnRH antagonists clearly suppress circulating LH levels, only minor decreases in FSH levels were detected using RIA. In men, immuno-FSH levels in serum decreased only 20% to 30%. We measured serum bio-FSH levels in four normal men after a single injection (20 mg) of a potent GnRH antagonist. Although only minimal suppression of immuno-FSH was detected, pronounced inhibition (79%–89%) of bio-FSH levels occurred (Fig 6; Dahl et al, 1988). Concomitantly, the ratio of bio- to immuno-FSH levels decreased drastically after the antagonist administration. These data reinforced earlier expectations that GnRH antagonists might be potential male contraceptives, and provided the first report of changes in circulating bio- to immuno-FSH levels.

*Molecular Basis for the Decreases in the B/I Ratios of FSH in GnRH Antagonist-treated Patients:* The chromatofocusing method separates proteins based on their isoelectric points. This technique has been shown to be useful in identifying changes in the microheterogeneity of FSH isoforms. We have set up this method, in conjunction with gel filtration (separation of proteins by molecular weight), to analyze FSH heterogeneity when changes in the B/I ratios of FSH are detected. The lower B/I ratios, and thus decreasing bio-potency of FSH, after GnRH antagonist injection may reflect the presence of different molecular species of

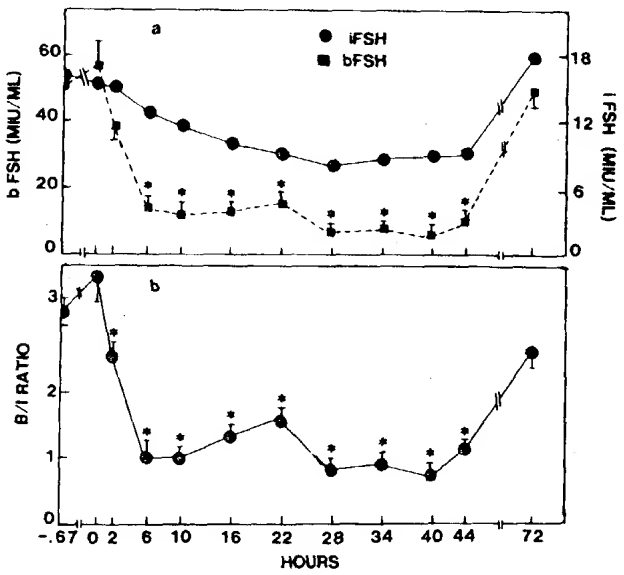


FIG. 6. Effects of GnRH antagonist treatment on bioactive and immunoreactive FSH in men. Effects of a subcutaneous injection of GnRH antagonist on bio(b)- and immuno(i)-FSH (a) and on the B/I ratio (b) in four normal men. Levels differing significantly from the pretreatment concentrations during treatment are marked with asterisks (*$P < 0.05$) (Dahl et al, 1988).

FSH. Therefore, we investigated the differences in FSH isoforms in postmenopausal women before and after GnRH antagonist treatment.

The FSH isoforms before and after GnRH antagonist treatment were separated based on their isoelectric properties using a chromatofocusing column with a pH gradient of 7 to 4 (Fig 7, Dahl et al, 1988). Each fraction was analyzed both by RIA (upper panel) and bioassay (lower panel). Four major hormone peaks were distinguished. Peak IV contained the majority of FSH isoforms based on both assays. Before treatment, peaks II and III contained the most bioactive FSH forms. After GnRH antagonist treatment, a substantial increase in the percentage of the most basic peak I was apparent, accompanied by decreases in the remaining peaks. The FSH B/I ratios were 0.1, 8, 57, and 2 for peaks I, II, III, IV, respectively, indicating that peak I was the least bioactive. The low B/I ratio in peak I suggested that these isoforms may be a mixture of FSH molecules with both agonist and antagonist activities.

*Identification of Circulating FSH Antagonist Isoforms:* To further characterize the FSH isoforms, additional chromatofocusing was performed (Dahl et al, 1988). To estimate the molecular weight (Mr) of the newly formed FSH isoforms, peak I fractions, obtained from GnRH antagonist-treated patients, were pooled and added to an Ultrogel (Pharmacia; Piscataway, NJ) column, which separates proteins by Mr. The applied sample displayed a wide range of Mr from 45,000 to 25,000, as assessed by RIA and the bioassay. However, the B/I ratios varied. Fractions with Mr and B/I ratios lower than intact FSH were pooled and run on

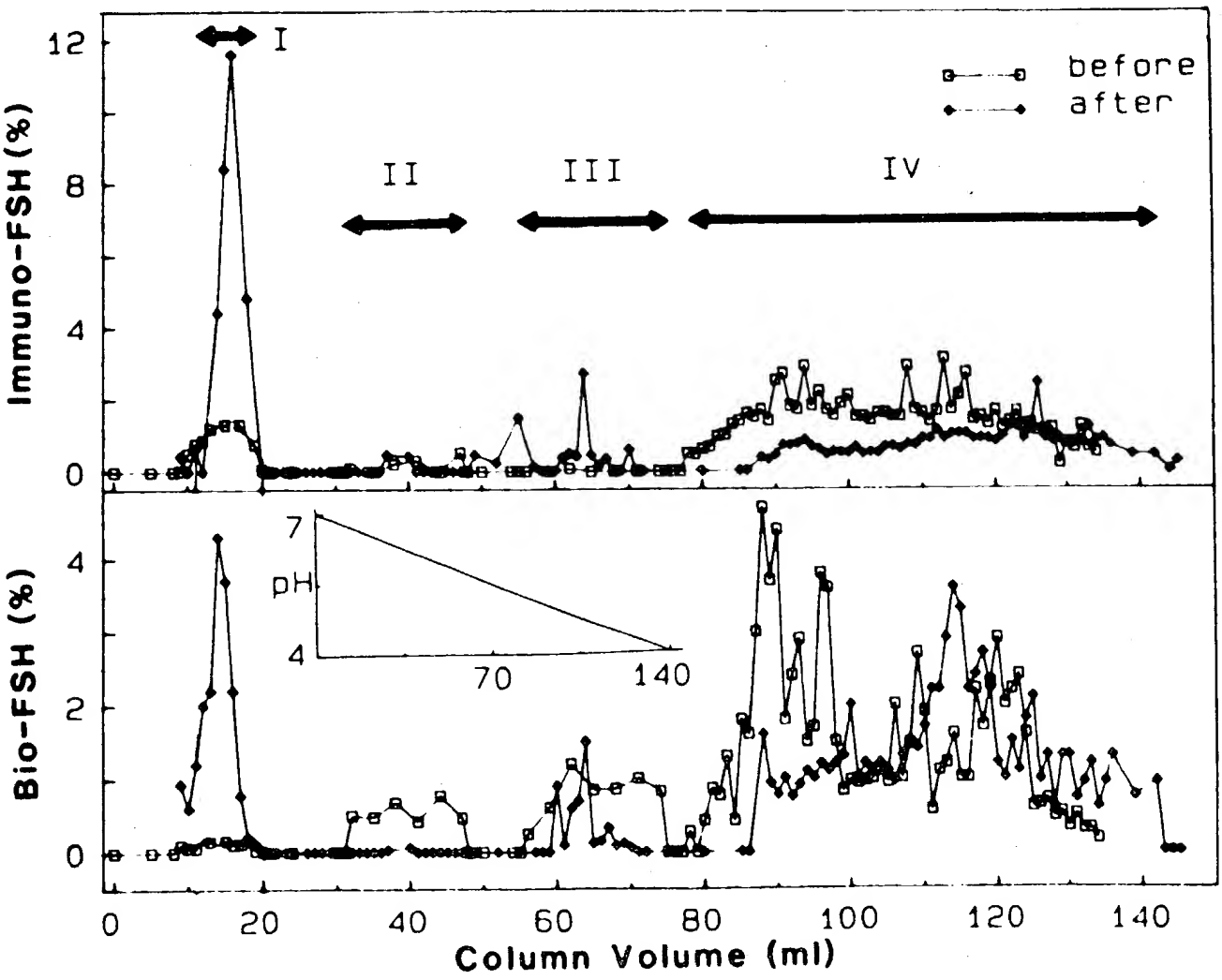


FIG. 7. Chromatofocusing analysis of serum samples from hypogonadal women before and after GnRH antagonist infusion. Chromatofocusing analysis of serum samples from hypogonadal women before (open squares) and after (closed diamonds) GnRH antagonist infusion, with the use of a pH gradient of 7 to 4 (inset). Fractionated samples were assayed (upper panel) by RIA, and (lower panel) by a granulosa cell aromatase bioassay. Results are expressed as the percent of total activity eluted from the column (Dahl et al, 1988).

a chromatofocusing column with a pH gradient from 11 to 7. The resulting fractions were again analyzed by RIA and bioassay (Fig 8, upper panel). Fractions from pH 9.6 to 9.4 contained the majority of the immuno-FSH, but these isoforms were not bioactive. To investigate possible antagonistic effects of these FSH isoforms, column fractions were added together with human FSH in the bioassay. Samples with pH 9.6 to 9.4 inhibited FSH-stimulated estrogen production (Fig 8, lower panel, open squares), but fractions with higher or lower pH were ineffective. A dose-dependent inhibition of estrogen production by the pH 9.5, but not by the pH 10.1, fraction was found (Fig 8, inset). To rule out nonspecific inhibitory effects, the same fractions were added in conjunction with forskolin, which bypasses the FSH receptor and directly activates adenyl cyclases to increase estrogen biosynthesis in the granulosa cells. There was no inhibition of forskolin-stimulated estrogen production by any fractions (Fig 8, lower panel; crosses), suggesting that the suppression of FSH action by these fractions is probably at steps before adenyl cyclase activation.

To further demonstrate that the antagonistic effect of the samples with pH 9.6 to 9.4 is due to FSH-like molecules, immunoneutralization experiments were performed. Treatment of cultured granulosa cells with antibodies against human FSH (Ab) or pooled fractions pH 9.6 to 9.4 (iso-FSH) alone did not increase estrogen production, whereas a near-saturating dose of ovine FSH increased estrogen production. Because the Ab is species-specific, no significant decrease in estrogen biosynthesis was detected when Ab was added in conjunction with ovine FSH. When the iso-FSH was added with ovine FSH, there was a significant inhibition of estrogen production. However, when Ab (1:1 or 1:5 dilutions) was added ovine FSH and the human iso-FSH, Ab blocked the inhibitory effects of iso-FSH in a dose-dependent manner. Therefore, the FSH antagonist isoforms were preferentially bound by the antibodies such that their receptor-specific inhibitory effects were blocked.

*GnRH Agonist Treatment in Men with Prostate Cancer:* To examine the effects of GnRH agonist treatment on FSH heterogeneity, bio- and immuno-FSH levels were measured in the serum of six patients with prostrate cancer during a 6-month treatment with a GnRH agonist analog (buserlin, 600 μg intranasally 3 times per day), and up to 12 weeks after subsequent orchidectomy (Fig 9; Huhtaniemi et al, 1988). After treatment with the agonist, both bio- and immuno-FSH transiently increased for 1 to 3 days. The increase in bioactivity was more pronounced and the B/I ratio

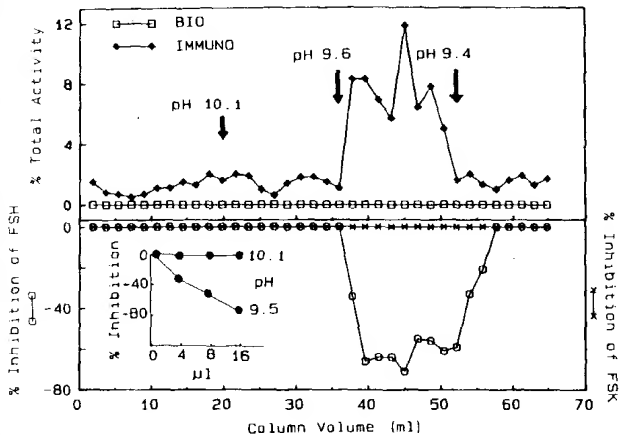


**FIG. 8.** Chromatofocusing analysis of GnRH antagonist-treated patient samples with the use of a pH gradient of 11 to 8. FSH isoforms with Mr and B/I ratio lower than those of intact FSH from an Ultrogel AcA 54 column were applied to the chromatofocusing column. **(A)** Column fractions were assayed by RIA, and by bioassay and shown as percent of total activity. **(B)** Antagonistic effects of FSH isoforms on FSH-stimulated (open squares) and forskolin-stimulated (crosses) estrogen production and dose-dependent inhibition of FSH-stimulated estrogen production by FSH isoforms (inset). Results were expressed as percent inhibition of estrogen production induced by FSH or forskolin (FSK) (Dahl et al, 1988).

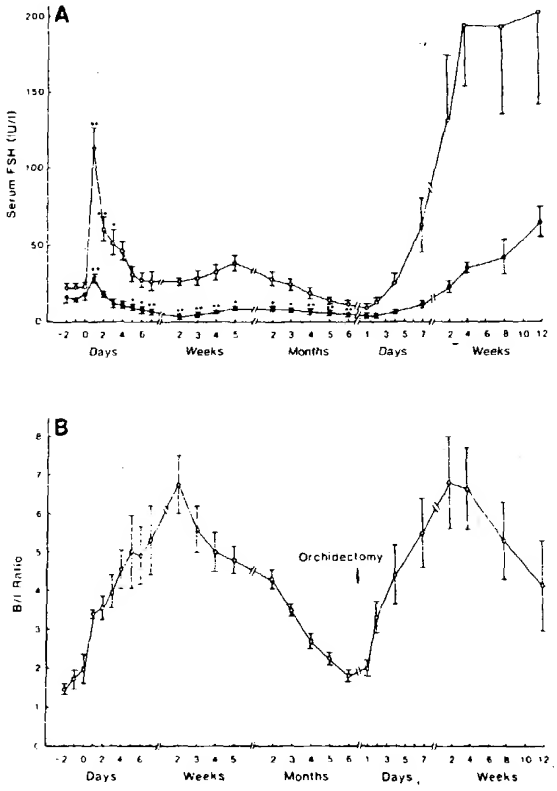


**FIG. 9.** Effects of GnRH agonist before and after orchidectomy in men with prostrate cancer. Serum bioactive (bio-) and immunoreactive (immuno-) FSH **(A)** levels and B/I ratios **(B)** were measured in patients treated for 6 months with the GnRH agonist buserlin, and for 12 weeks following orchidectomy at 6 months after the initiation of agonist therapy. Levels differing significantly from the pretreatment concentrations during the peptide treatment are marked with asterisks (*$P < 0.05$) (Huhtaniemi et al, 1988).

increased at 2 weeks (Fig 9). During later stages of treatment, immuno-FSH declined below pretreatment levels in 5 days, and stayed suppressed for the rest of the treatment. In contrast, FSH bioactivity did not decrease significantly below the treatment level during the 6-month treatment period, although the B/I ratio returned slowly toward pretreatment levels. After orchidectomy, the FSH activities showed dramatic increases, and the B/I ratio rose transiently from 1.5 to 7 in 2 weeks. The bio-FSH levels increased more acutely than immuno-FSH after GnRH agonist treatment and orchidectomy. A clear decline during long-term agonist treatment is only seen in immuno-FSH, which contrasts to the concomitant decline in serum LH bioactivity reported earlier. When samples with high and low FSH B/I ratios were subjected to chromatofocusing (Fig 10), there was a shift from more acidic isoforms (low B/I ratio) to more basic isoforms (high B/I ratios). The persistence of FSH bioactivity may partly explain the inconsistent effects of GnRH agonist treatments on the suppression of spermatogenesis.

### *Testosterone-treated Men*

In normal men, chronic administration of high doses of testosterone markedly suppresses FSH and LH by RIA and reduces sperm production, but consistent azoospermia is not achieved (Matsumoto, 1990; Morse et al, 1973; Paulsen et al, 1982). To determine whether testosterone differentially affects the gonadotropin bioactivities, and whether these alterations result in inconsistent azoospermia, we studied normal men after a 4- to 6-month control, treatment, or recovery period. Parallel dose-dependent decreases in both bio- and immuno-LH occurred during testosterone treatment (Fig 11). However, LH B/I ratios did not change significantly. Chromatofocusing of serum samples from men before and after testosterone treatment (Fig 12) indicated a change in the microheterogeneity from more acidic isoforms with B/I ratios of approximately 1 to more bioactive FSH isoforms with pHs approximately 5.9 and 6.6 (B/I ratios 2 to 4, respectively). In conclusion, these findings demonstrate that the administration of testosterone to normal men results in dose-dependent decreases in both bio- and immuno-FSH and LH, unchanged B/I ratios of LH, increased FSH B/I ratios, and a shift from more acidic to more basic and bioactive FSH isoforms with high doses of testosterone. Therefore, persistence of circulating bioactive

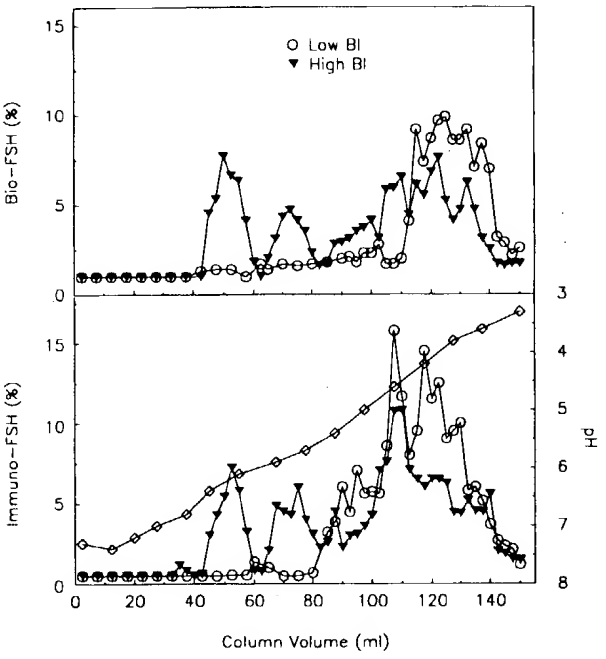


**FIG. 10.** Chromatofocusing analysis of serum samples from GnRH agonist-treated men before and after orchidectomy. Chromatofocusing analysis of serum samples before (open circles) and after (closed triangles) orchidectomy, with the use of a pH gradient of 7 to 4 (inset). Fractionated samples were assayed by RIA (*upper panel*), and by a granulosa cell aromatase bioassay (*lower panel*). Results are expressed as the percent of total activity eluted from the column.

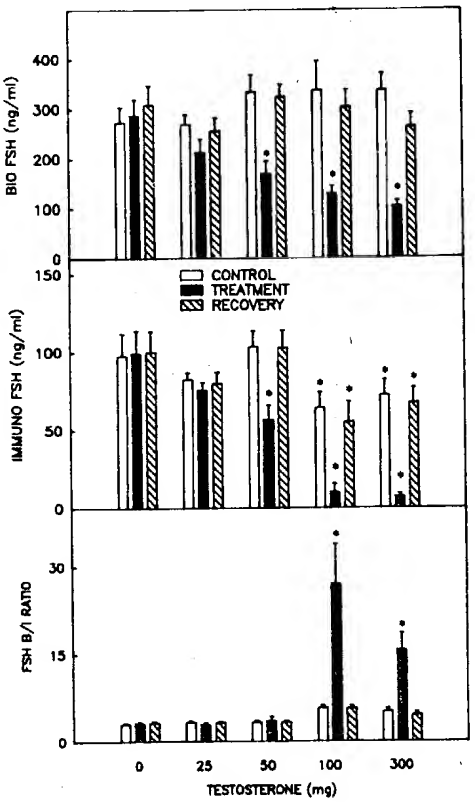


**FIG. 11.** Effects of testosterone treatment on bioactive and immunoreactive FSH levels. Serum bioactive (bio-) (*upper panel*) and immunoreactive (immuno-) (*middle panel*) FSH levels and B/I ratios (*lower panel*) were measured in patients treated for 6 months with testosterone. Levels differing significantly from the pretreatment concentrations treatment are marked with asterisks (*$P < 0.05$).

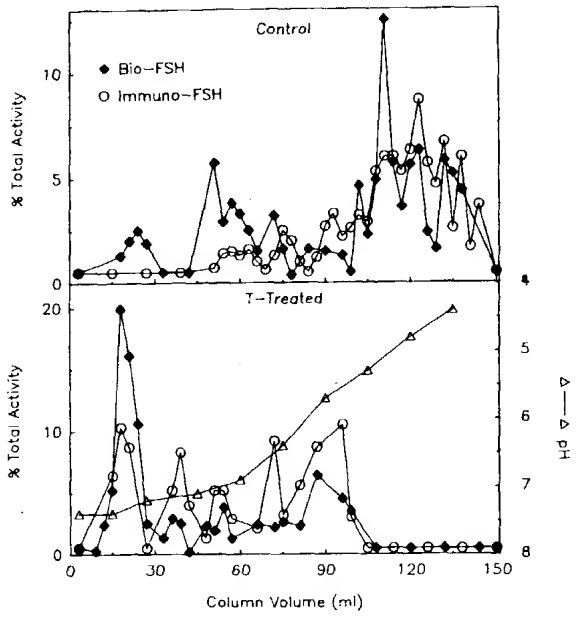


**FIG. 12.** Chromatofocusing analysis of serum samples before and after testosterone treatment. Chromatofocusing analysis of serum samples before (*upper panel*) and after (*lower panel*) testosterone treatment, with the use of a pH gradient of 7 to 4 (inset). Fractionated samples were assayed by RIA (open circles), and by a granulosa cell aromatase bioassay (closed diamonds). Results are expressed as the percent of total activity eluted from the column.

gonadotropins may contribute to the lack of achievement of consistent azoospermia during high-dose testosterone administration in normal men.

## Conclusion

*In vitro* assays provide the tool to measure FSH levels in diverse physiologic and pharmacologic states. Observed differences in bio- and immuno-FSH and LH secretion suggest that separate structural entities are recognized by the bioassays versus the immunoassays, and that RIA does not consistently provide a good estimate of bioactive gonadotropin levels. We observed increases in the B/I ratio of FSH in GnRH agonist-treated patients with prostate cancer and testosterone-treated normal men, and decreases in the B/I ratio after GnRH antagonist treatment in both men and women. Chromatofocusing analysis of serum from GnRH antagonist-treated patients demonstrates a naturally occurring circulating "anti-hormone," and provides the basis to elucidate the role of hormone antagonists in various physiologic, pharmacologic, and pathophysiologic states. Further characterization and eventual purification of these FSH isoforms could aid our understanding of the glycosylation and action of FSH, as well as provide clinical approaches for "anti-hormone" therapies. In addition to GnRH antagonists and testosterone, the observed FSH antagonist isoforms may provide prototypes for the design of contraceptives.

## References


Albert A, Rosemberg E, Ross GT, Paulsen CA, Ryan RJ. Report of the National Pituitary Agency collaborative study on the radioimmunoassay of FSH and LH. *J Clin Endocrinol Metab*. 1968;28:1214–1219.

Ax RL, Ryan RJ. FSH stimulation of 3H-glucosamine incorporation into proteoglycans by porcine granulosa cells in vitro. *J Clin Endocrinol Metab*. 1979;49:646–648.

Bahl OP, Thotakura NR, Anumula KR. In: BB Saxena et al, eds. *Hormone Receptors in Growth and Reproduction*. New York: Raven Press; 1984:165–183.

Baenziger JU, Green ED. Pituitary glycoprotein hormone oligosaccharides: structure, synthesis and function of the asparagine-linked oligosaccharides on lutropin, follitropin, and thyrotropin. *Biochim Biophys Acta*. 1988;947:287–306.

Beers WH, Strickland S, Reich E. A cell culture assay for follicle-stimulating hormone. *J Biol Chem*. 1978;253:3877–3884.

Blum WFP, Gupta D. Heterogeneity of rat FSH by chromatofocusing: studies on serum FSH, hormone released in vitro and metabolic clearnace rates of its various forms. *J Endocrinol*. 1985;105:29–37.

Bogdanove EM, Campbell GT, Blair ED, Mula ME, Miller AE, Grossman GH. Gonad-pituitary feedback involves qualitative change: androgens alter the type of FSH secreted by the rat pituitary. *Endocrinology*. 1974;95:219–228.

Brown PS, Wells M. Observations on the assay of human urinary follicle-stimulating hormone by the augmentation test in mice. *J Endocrinol*. 1966;35:199–206.

Calvo FO, Keutmann HT, Bergert ER, Ryan RS. Deglycosylated human follitropin: characterization and effects on adenosine cyclic 3', 5' -phosphate production in porcine granulosa cells. *Biochemistry*. 1986; 25:3938–3943.

Catt KJ, Dufau ML, Tsuruhara J. Absence of intrinsic biological activity in LH and hCG subunits. *J Clin Endocrinol Metab*. 1972;34:1972–1976.

Chappel SC. The presence of two species of follicle-stimulating hormone within hamster anterior pituitary glands as disclosed by concanavalin A chromatography. *Endocrinology*. 1981;109:935–942.

Chappel SC, Ulloa-Aguirre A, Coutifaris C. Biosynthesis and secretion of follicle-stimulating hormone. *Endocrinol Rev*. 1983a;4:179–211.

Chappel SC, Ulloa-Aguirre A, Ramaley JA. Sexual maturation in female rats: time-related changes in the isoelectric focusing pattern of anterior pituitary follicle-stimulating hormone. *Biol Reprod*. 1983b;28:195–205.

Cheng KW. A radioreceptor assay for follicle-stimulating hormone. *Clin Endocrinol Metab*. 1975;41:581–589.

Crowley, WF JR, Filicori M, Spratt D, Santoro N. The physiology of gonadotrophin-releasing hormone (GnRH) secretion in men and women. *Recent Prog Horm Res*. 1985;41;473.

Dahl KD, Hsueh AJW. Mechanism of action of FSH in the ovary. In: Cook B, King R, Van Der Molen H, eds. *New Comprehensive Biochemistry: Hormones and Their Action*. Amsterdam, The Netherlands: Elsevier Science Publishers; 1988:181–192.

Dahl KD, Jia XC, Hsueh AJW. Granulosa cell aromatase bioassay for follicle-stimulating hormone. *Methods Enzymol*. 1989a;168:414–423.

Dahl KD, Papkoff H, Hsueh AJW. Effect of diverse mammalian gonadotropins on estrogen production in a sensitive granulosa cell aromatase bioassay (GAB). *Gen Comp Endocrinol*. 1989b;73:368–373.

Dahl KD, Bicsak TA, Hsueh AJW. Naturally occurring "anti-hormones":

secretion of FSH antagonist isoforms by hypogonadal women treated with a GnRH analog. *Science*. 1988;239:72–74.

Diebel ND, Yamamoto M, Bogdanove EM. Discrepancies between radioimmunoassays and bioassay for rat FSH. Evidence that androgen treatments and withdrawal can alter bio-assay-immunoassay ratios. *Endocrinology*. 1973;92:1065–1078.

Faiman C, Ryan KJ. Radioimmunoassay for human follicle stimulating hormone. *J Clin Endocrinol Metab*. 1967;77:444–447.

Galle PC, Ulloa-Aguirre A, Chappel SC. Effects of estradiol, phenobarbitone, and lutenizing hormone releasing hormone upon the isoelectric profile of pituitary follicle-stimulating hormone in ovariectomized hamsters. *J Endocrinol*. 1983;99:31–39.

Gharib SD, Wierman ME, Shupnik MA, Chin WW. Molecular biology of the pituitary gonadotropins. *Endocr Rev*. 1990;11:177–199.

Hsueh AJW, Liu Y-X, Cajander SB, et al. Gonadotropin releasing hormone induces ovulation in hypophysectomized rats: Studies on ovarian tissue-type plasminogen activator activity, messenger ribonucleic acid content and cellular localization. *Endocrinology*. 1988;122:1486–1495.

Huhtaniemi IT, Dahl KD, Rannikko S, Hsueh AJW. Serum bioactive and immunoreactive follicle-stimulating hormone in prostatic cancer patients during GnRH agonist treatment and after orchidectomy. *J Clin Endocrinol Metab*. 1988;66:361–370.

Jockenhovel F, Khan SA, Nieschlag E. Varying dose-response characteristics of different immunoassays and *in vitro* bioassay for FSH are responsible for changing ratios of biologically active to immunologically active FSH. *J Endocrinol*. 1990;127:523–532.

Keutmann HT, Johnson L, Ryan RJ. Evidence for a conformational change in deglycosylated glycoprotein hormones. *FEBS Lett*. 1982;185:333–338.

Kornfield R, Kornfield S. Assembly of asparagine-linked oligosaccharides. *Ann Rev Biochem*. 1985;54:631–664.

Labrie F, Dupont A, Belander A, et al. Treatment of prostate cancer with gonadotrophin-releasing hormone agonists. *Endocrinol Rev*. 1986;7:67.

Limbird LE. Activation and attenuation of adenylate cyclase. *Biochem J*. 1981;195:1–13.

Linde R, Doelle GC, Alexander N, et al. Reversible inhibition of testicular steroidogenesis and spermatogenesis by a potent gonadotropin-releasing hormone agonist in normal men: an approach toward the development of a male contraceptive. *N Engl J Med*. 1981;305:663.

Ling N, Ying S-Y, Ueno N, et al. Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. *Nature*. 1986;321:779.

Liu WK, Young JD, Ward DN. Deglycosylated ovine iutropin: preparation and characterization by in vitro binding and steroidogenesis *Mol Cell Endocrinol*. 1984;37:29–39.

Manjunath P, Sairam MR, Sairam J. Studies on pituitary follitropin. X. Biochemical, receptor binding and immunological properties of deglycosylated ovine hormone. *Mol Cell Endocrinol*. 1982;28:125–138.

Matsumoto AM. Effects of chronic testosterone administration in normal men: safety and efficacy of high dosage testosterone and parallel dose-dependent suppression of LH, FSH, and sperm production. *J Clin Endocrinol Metab*. 1990;70:282–287.

McIlroy PJ, Ryan RJ. Mechanism of action of gonadotrophins. In: Li CH, ed. *Hormonal Proteins and Peptides*. New York: Academic Press; 1983:93–133.

McLachlan RI, Robertson DM, Healy DL, Burger HG, de Kretser DM. Recombinant human activin-A stimulates basal FSH and GnRH-stimulated FSH and LH release in the adult male macaque, Macaca fascicularis. *J Clin Endocrinol Metab*. 1987;65:954–961.

Means AR, Dedman JR, Tash JS, Tindall DJ, Van Sickle M, Welsh MJ. Regulation of the testis sertoli cell by FSH *Ann Rev Physiol*. 1980;42:59–70.

Midgley AR, Jr. Radioimmunoassay for human follicle-stimulating hormone. *J Clin Endocrinol Metab*. 1967;27:295.

Midgley AR Jr. Autoradiographic analysis of gonadotrophin binding to rat ovarian tissue sections. *Adv Exp Med Biol*. 1973;36:365–378.

Midgley AR Jr, Jaffe RB. Regulation of human gonadotrophins IV: correlation of serum concentrations of follicle-stimulating and luteinizing hormone during the menstral cycle. *J Clin Endocrinol Metab*. 1968;28:1699–1703.

Morse HC, Horike M, Rowley MJ, Heller CG. Testosterone concentrations in testes of normal men: effects of testosterone propionate administration. *J Clin Endocrinol Metab*. 1973;37:882–886.

Moyle WR, Erickson G, Bahl OP, Chiiotukoo S, Gutowski J. Action of PMSG and asialo-PMSG on rat Leydig and granulosa cells. *Am J Physiol*. 1978;235:E218–E227.

Ny T, Bjersing L, Hsueh AJW, Loskutoff DJ. Cultured granulosa cells produce two plasminogen activators, each regulated differently by gonadotrophins. *Endocrinology*. 1985;116:1666–1668.

Padmanabhan V, Chappel SC, Beitins IN. An improved *in vitro* bioassay for follicle-stimulating hormone (FSH): suitable for measurement of FSH in unextracted human serum. *Endocrinology*. 1987;121:1089–1098.

Papkoff H, Sairman MR, Farmer SW, Li CH. Studies on the structure and function of interstitial cell-stimulating hormone. *Rec Prog Horm Res*. 1973;29:563–592.

Paulsen CA, Bremner WJ, Leonard JM. Male contraception: clinical trials. In: Mishell DR Jr, ed. *Advances in Fertility Regulation*. New York: Raven Press; 1982:157–170.

Pavlou SN, Interlandi JW, Wakefield G, Rivier J, Vale W, Rabin D. Heterogeneity of sperm density profiles following 16-week therapy with continuous infusion of high-dose LHRH analog plus testosterone. *J Androl*. 1986;7:228.

Peckham WD, Knobil E. Qualitative changes in the pituitary gonadotrophins of the male rhesus monkey following castration. *Endocrinology*. 1976;98:1054–1060.

Peckham WD, Yamaji T, Dierschke DJ, Knobil E. Gonadal function and the biological properties of follicle stimulating hormone. *Endocrinology*. 1973;92:1660–1666.

Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. *Ann Rev Biochem*. 1981;50:465.

Ramachandran J. In vitro methods of gonadotropin assays. In: Li CH, ed. *Hormonal Proteins and Peptides*. New York: Academic Press; 1983:1–79.

Reichert LE, Bhalla VK. Development of a radioligand tissue receptor assay for human follicle stimulating hormone. *Endocrinology*. 1974;94:483–491.

Reichert LE, Dattatreyamurty B, Grasso P, Santa-Coloma TA. Structure-function relationships of the glycoprotein hormones and their receptors. *Trends Pharmacolog Science*. 1991:199–203.

Rivier C, Rivier J, Vale W. GnRH Antagonist: physiologic and contraceptive applications in the female rat. In: Zatichni GI, Shelton JD, Sciarra JJ, eds. *Contraceptives*. Philadelphia: Harper and Row; 1981:13.

Rosenberg E, Solod EA, Albert A. Luteinizing hormone activity of the human pituitary gonadotropin as determined by the ventral prostate weight and the ovarian ascorbic acid depletion methods of assay. *J Clin Endocrinol Metab*. 1971;24:714–728.

Ross EM, Gilman AG. Biochemical properties of hormone-sensitive adenylate cyclase. *Ann Rev Biochem*. 1980;49:533–564.

Ross GT, Cargille GM, Lipsett MB Rayford PL, Marshall JR, Strott CA, Rodbard D. Pituitary and gonadal hormone in women during spontaneous and induced ovulation cycles. *Recent Prog Horm*. 1970;26:1–62.

Ryan RJ. A comparison of biologic and immunologic potency estimates of

human lutenizing and follicle stimulating hormones. *Acta Endocrinol [Suppl] (Copenh).* 1969;12:300–324.

Ryan RJ, Faiman C. Proceedings of the informational symposium on protein and polypeptide hormones. *International Congress Series-Excerpta Med.* 1968;161:129–146.

Ryan RJ, Keutmann, HT, Charlesworth MC, et al. Structure function relationships of gonadotropins. *Recent Prog Horm.* 1987; 43:383–423.

Ryle M, Chaplain MF, Gray CJ, Kennedy JF. In: Butt WR, Crooke AC, Ryle M, eds. *Gonatropins and Ovarian Development.* London: Livingston; 1970:98–106.

Sairam JR, Manjunath P. Studies on pituitary follitropin. XI. Induction of hormonal antagonistic activity by chemical deglycosylation. *Mol Cell Endocrinol.* 1982;28:125–138.

Sairam MR. Gonadotrophic hormones. In: Li CH, ed. *Hormonal Proteins and Peptides.* New York: Academic Press; 1983:1–79.

Saxena BB, Rathnam P. Amino acid sequence of the β subunit of FSH from human pituitary glands. *Biol Chem.* 1976;251:993–1002.

Simpson ME, Evans HM, Li CH. Growth of hypophysectomized female rats following chronic treatment with pure pituitary growth hormone; general growth and organ changes. *Anat Rec.* 1950;106:247.

Sluss PM, Schneyer AL, Franke MA, Reichert LE Jr. Porcine follicular fluid contains both follicle-stimulating hormone agonist and antagonist activities. *Endocrinology.* 1987;120:1477–1481.

Steelman SL, Pohley FM. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotrophin. *Endocrinology.* 1953;53:604–616.

Taymore ML, Miyata J. Discrepancies and similarities of serum FSH and LH patterns as evaluated by different assay methods. *Acta Endocrinol [Suppl] (Copenh).* 1969;142:324–357.

Uberoi NK, Meyer RK. Uterine weight of the immature rat as a measure of augmentation of pituitary gonadotrophins by human chorionic gonadotrophin (HCG). *Fertil Steril.* 1967;48:420–428.

Ulloa-Aguirre A, Miuer C, Hyland L, Chappel S. Production of all follicle-stimulating hormone isohormones from a purified preparation by neuraminidase digestion. *Biol Reprod.* 1984:30;382–387.

Ulloa-Aguirre A, Mejia JJ, Dominguez R, Guevara-Aguirre J, Diaz-Sanchez V, Larra F. Microheterogeneity of anterior pituitary FSH in the male rat: isoelectric focusing pattern throughout sexual maturation. *Endocrinology.* 1986;110:539–549.

Vaitukaitis JL, Ross GT, Braunstein GD, Rayford PL. Gonadotropin and their subunits: basic and clinical studies. *Rec Prog Horm Res.* 1976; 32:289–295.

Vale W, Rivier J, Vaughan J, et al. Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. *Nature.* 1986;321:776.

Van Damme MP, Robertson DM, Marano R, Ritzen EM, Diczfalusy E. A sensitive and specific in vitro bioassay method for the measurement of follicle-stimulating hormone activity. *Acta Endocrinol (Copenh).* 1979;91:224–237.

Vickery BH. Comparisons of the potential utility of LHRH agonists and antagonists for fertility control. *J Steroid Biochem.* 1985;23:779.

Wang C. Bioassays of follicle stimulating hormone. *Endocrinol Rev.* 1988;9:374–377.

Wang C, Leung A. Gonadotropins regulate plasminogen activator production by rat granulosa cells. *Endocrinology.* 1983;112:1201–1207.

Welsh TH Jr, Jia X-C, Hsueh AJW. Forskolin and phosphodiesterase inhibitors stimulate rat granulosa cell differentiation. *Mol Cell Endocrinol.* 1984;37:51–60.

Wide L. Male and female forms of follicle-stimulating hormone in serum. *J Clin Endocrinol Metab.* 1982;55:682–688.

Wide L. Median charge and heterogeneity of human pituitary FSH and LH. II. Relationship of sex and age. *Acta Endocrinol (Copenh).* 1985; 109:190–197.

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